

## TRANSVAGINAL OVUM PICK-UP (OPU) IN THE COW: A NEW DISPOSABLE NEEDLE GUIDANCE SYSTEM

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### ABSTRACT

A study was conducted to modify the routinely used ovum pick up (OPU) devices to permit use of disposable needles and to simplify the technique and to make it more economical and practical to use. Long nondisposable needles are commonly used in transvaginal OPU despite several disadvantages. A new OPU device was developed using 19-g disposable needles to eliminate these disadvantages and to make the technique more successful. The disposable needle was connected to silicone tubing by means of a stainless steel connector. The system was inserted into a stainless steel tube, creating a rigid structure within which to move the needle back and forth. A blunt needle can be changed simply by replacing it with a new one, even while the device is in the vagina of a cow. The needle guidance system is incorporated into a new OPU device together with the transducer of an ultrasonographic scanner with an unilateral orientated scanning field, making it possible to utilize the needle length to its maximum. This combination permits easy manipulation of the ovaries, easy positioning of follicles on the puncture line, and enables the use of shorter needles which directly enter the scanned area without loss of useful needle length. As a preliminary result we obtained an overall oocyte recovery rate of 42%. Although this is promising, additional puncture sessions are needed to establish more consistent recovery rates. When OPU is used routinely, application of short disposable needles is more practical and economical.

Key words: transvaginal ovum pick-up, bovine reproduction, disposable needle guidance system

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## INTRODUCTION

In recent years, several new techniques have been introduced in bovine reproduction and with the initiation of in vitro fertilization (IVF) programs, a need for good quality oocytes arose. Traditionally, these oocytes were obtained from slaughterhouse material, the ovaries being collected at slaughter and transported to the laboratory. All follicles (2 to 5 mm) visible on the ovarian surface were punctured and the oocytes selected and cultured in maturation medium. Although this technique has been refined considerably, there are still some disadvantages: 1) the time between collecting the oocytes and placing them into the culture medium was, in some cases, too long; 2) nothing was known about the hormonal status or health of the slaughtered animals; and 3) the lack of repeatability of this technique in infertile patients, often genetically superior donors. As there can be no further collection of oocytes once the cow has been slaughtered, numerous researchers have looked for techniques to retrieve oocytes from the living cow. In the first experiments laparoscopy and laparotomy were used to reach the ovaries (5, 6), later, laparoscopy was combined with ultrasonography to make the recovery of oocytes less invasive (2). A breakthrough was achieved with the adoption of human transvaginal ultrasound scanning procedures for ovum pick-up (OPU) in the bovine (9). This technique has several advantages: it is less traumatic for the animals and less invasive than other systems, and it has a high degree of repeatability (10, 12, 14).

After the initial adaptation of OPU to the bovine, several groups have tried to increase the number of oocytes retrieved. Improvements in recovery rates were obtained by modifying both the pretreatment of the animals to be punctured and the frequency of puncturing (3, 8, 11, 12, 14, 17). Recently, technical details of the OPU apparatus have become a major subject for investigators. Rath (personal communication) developed an OPU system which uses disposable needles, while Scott et al. (1994) described the use of different types of scanners and developed a new needle guidance system using long nondisposable needles. At present, 3 types of needles are in use: long nondisposable needles, long semi-disposable needles and fully disposable needles. The first are still commonly used (4,7,9) although they have several disadvantages. They dull very quickly, and a resharpened needle never regains its initial sharpness (14). Long nondisposable needles also make it difficult to collect follicular fluid from individual follicles due to the large dead volume (Vos, personal communication), and, these needles are quite expensive. Long semi-disposable needles consist of 2 parts, a long fine and hollow stainless steel tube to the end of which the stainless steel part of a disposable needle is attached, after the hub has been removed. When the needle becomes dull, only the small disposable part needs to be replaced, which requires a specialized technician, thus again resulting in an expensive needle. (Vos, personal communication) Recently, fully disposable needles were introduced by Rath (personal communication), which are easy and inexpensive to replace, and permit the use of a new needle for each patient. The disposable needles are short and easy to manipulate, the dead volume is small and the needles are very well suited for collecting follicular fluid from individual follicles.

This paper describes the development of a new simplified OPU device combining a multiple angle mechanical sector probe (MAP) with an unilateral scanning area and a new disposable needle guidance system using short disposable 19-g needles.

## MATERIALS AND METHODS

### Animals

Twenty-seven healthy, multiparous cows of different Belgian breeds with normal reproductive tracts were used. The cows were fed a mixed ration consisting of hay and a commercial concentrate pellet. They were housed in tie-stalls at the Department of Obstetrics, Reproduction and Herd Health, Faculty of Veterinary Medicine, Ghent, Belgium. They received no hormonal pretreatment except in those cases in which ovarian activity was found to be low upon echographic examination, when the cows were stimulated with FSH/LH (Stimufol®, Rhone Mérieux) for 4 consecutive days, using 8 equal doses for a total dose of 500 µg pFSH and 100 µg pLH. Cows showing a mature corpus luteum with echography image were treated with 500 µg cloprostenol (Estrumate®, Pitman-Moore). The puncturing of the follicles started on Days 5 to 6 of the estrus cycle and continued twice a week for the following 2 wks for some of them. Animals were sedated using detomidine hydrochloride 10 mg/ml iv (Domosedan®, Smith Kline Animal Health Ltd) at a dose of 0.1 cc /100 kg bodyweight which in addition to its sedative effect also makes transrectal manipulation of the ovaries easier by relaxing the intestines. Additional relaxation was obtained by epidural anesthesia using 5 ml of 2% lidocaine. Oocytes were collected in plastic 50-ml collecting tubes (Falcon 2074) containing phosphate buffered saline (PBS) and heparin (125,000 IU/l) and were observed under a stereomicroscope. They were then individually transferred, within 10 min, to a 50-µl droplet of maturation medium overlaid with paraffin oil. Finally, the oocytes were cultured at 39 C° in 5% CO<sub>2</sub> in air with maximal humidity, following standard IVF procedures (16).

### OPU Equipment

A new needle guidance system was designed using 19-g disposable, single lumen needles (Terumo NEOLUS 19GX2" 1.1X50) (Figure 1). In order to move within the stainless steel guidance tube at the inner part of the device, the luer-lock hub of the needle was shortened by 1 mm, removing the widest part. The needle was then attached by means of a stainless steel connector to silicone tubing (Silclear TM Tubing, Multi Purpose Medical Grade Silicone Tubing, Degania Silicone/Israel) which was passed through a stainless steel tube (length 60 cm, diameter 6 mm). The various diameters of the connecting piece, the stainless steel tube (6 mm) and the silicone tubing (4 mm) caused the silicone tubing to be trapped between the stainless steel parts when the needle is drawn back into the stainless steel tube, creating a rigid structure which permitted the needle to be moved back and forth. A blunt needle can be changed simply by removing it and replacing it with a new one. Before inclusion in the system, the silicone tubing was tested for oototoxicity (1). This was carried out by culturing oocytes in maturation medium which had been placed in the tubing overnight (24 h). No toxic effects were noted.

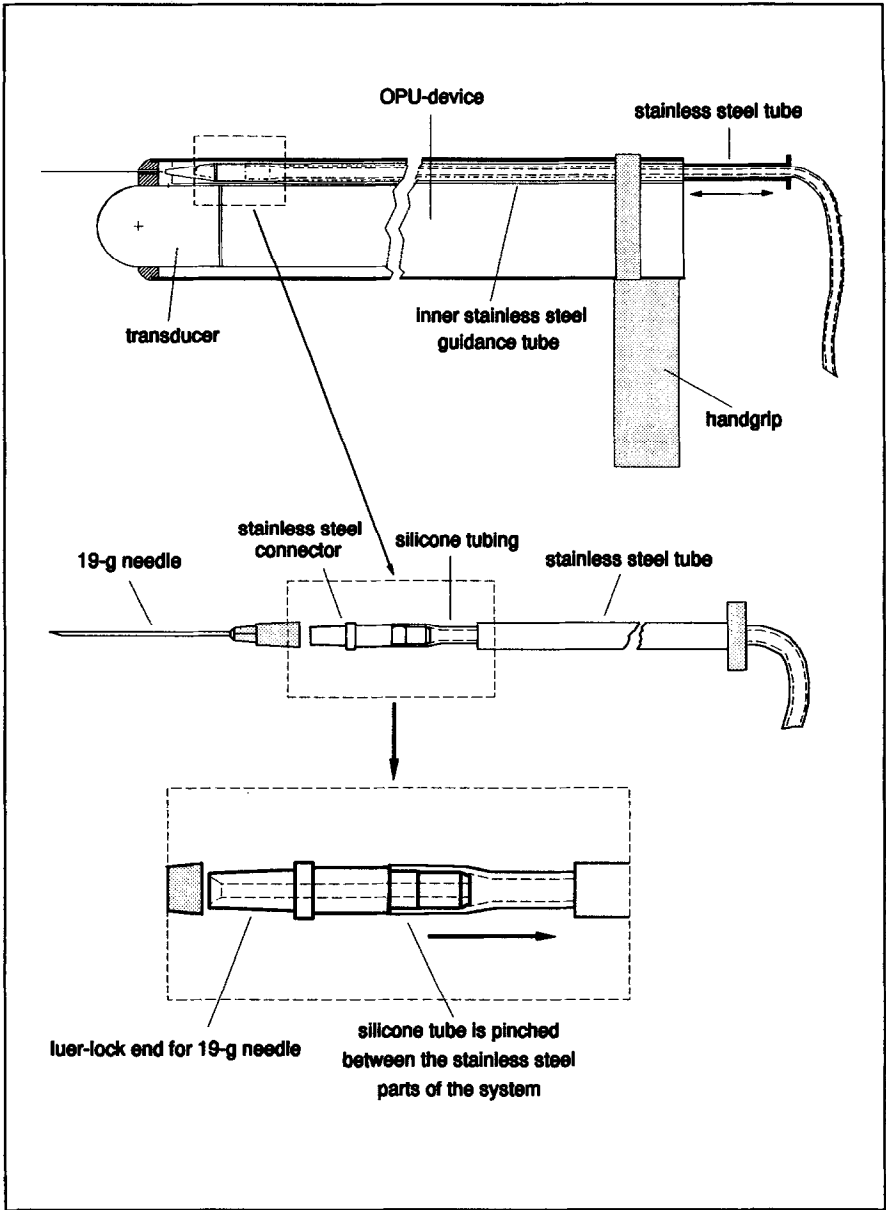


Figure 1. Needle guidance system.

This needle guidance system was incorporated into a new OPU device alongside the transducer of an ultrasonographic scanner (Pie Medical Scanner 200 vet). The use of an inner stainless steel guidance tube (an 8 mm diameter) had important implications for the scanning field requirements, as the needle's axis moved further away from the center of the transducer due to the large diameter, as shown in Figure 2. The useful length of the needle is shortened by the portion that does not enter the scanned area. There are 2 possibilities to overcome this problem: either the use of longer needles (80 to 100 mm), or the use of a scanner with a larger, unilateral scanning field. A mechanical multi angle probe (MAP) transducer (Pie Medical) with an extended ultrasound examination field at an angle of 150 degrees, several orientation possibilities and a frequency of 7.5 MHz was chosen over longer needles for stability and because of trauma to the ovaries that longer needles could cause. This extension of the examination field clearly permits easy manipulation of the ovaries and positioning of a follicle on the puncture line. The combination of the two systems enables the use of shorter needles which directly enter the scanned area, without loss of useful needle length.

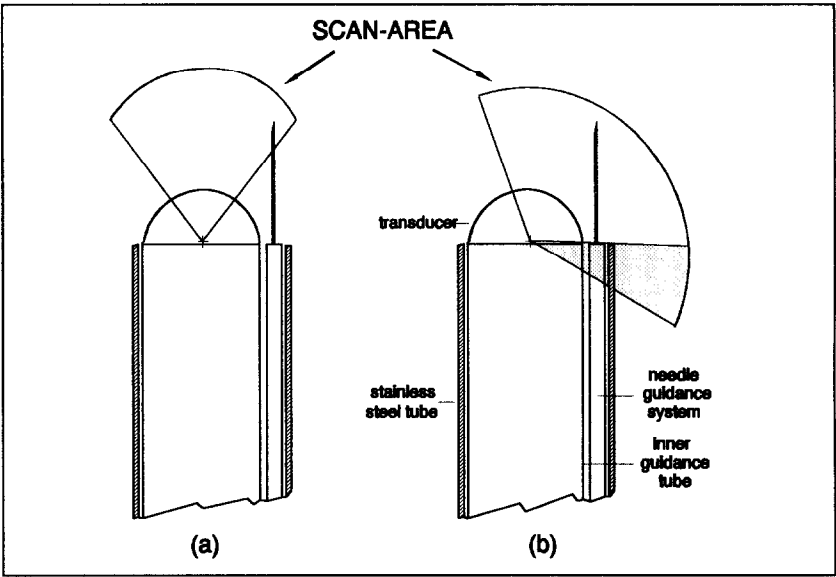


Figure 2. A forward-orientated ultrasonic field at an angle of 90 degrees in combination with a disposable-needle guidance system causes a long distance between the needle's axis and the head of the transducer (a). As a consequence the useful needle length is shortened. To overcome this problem an expanded ultrasound examination field can be used (b).

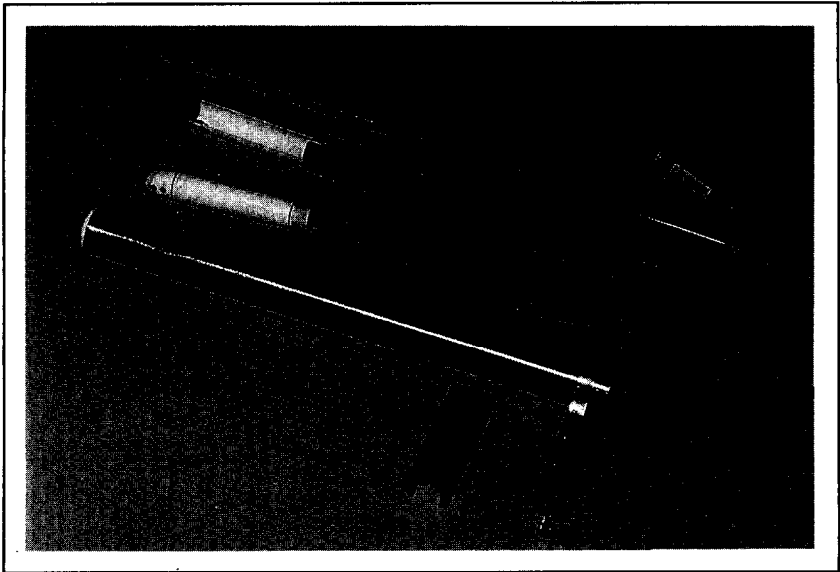


Figure 3. OPU device with the new needle guidance system.

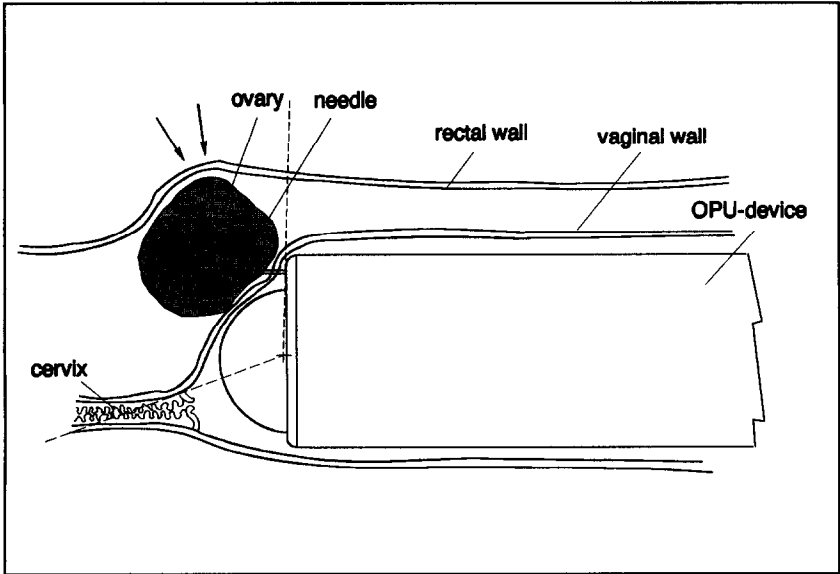


Figure 4. Positioning of the ovary.

The transducer and the needle guidance system are inserted into a stainless steel holder with a diameter of 45 mm suitable for use in both heifers and cows (Figure 3), while the length of the total system permits its use in the mare. The handgrip is designed to allow the operator to fix the OPU device while it is placed in the vagina, by gently pressing the handgrip with the right side of the body, leaving the left hand free to manipulate the needle.

To create a vacuum, a suction pump with variable suction power (Labotech) was used and preliminary results were obtained with suction powers between 90 and 100 mm Hg, corresponding to an actual suction power of 36 ml/min of water.

The top of the transducer is covered with contact-gel and a sterile plastic cap is placed over the OPU device before insertion into the vagina. The tubing is filled with collecting medium before the puncture session begins. If necessary, the needle can be changed while the device is placed in the vagina.

Figure 4 shows the positioning of the ovary. The head of the OPU device becomes asymmetric due to the use of the extended needle guidance system, which creates a ridge on which the ovary can be easily fixed against the head of the transducer, eliminating the problem of keeping the ovary in the middle of the ultrasonic field. The combination with the unilateral scanning area gives a high quality image because of the close contact between the ovary and the transducer.

## RESULTS

Preliminary results are shown in table 1. A total of 27 different cows was used. Some cows were only punctured once, while others were punctured twice a week for 3 consecutive weeks. In total, 291 follicles were aspirated and 122 oocytes were recovered for a recovery rate of 42%. The initial recovery rates were inconsistent, due to the operator's lack of experience. The quality of the oocytes was determined by maturation and culture using the normal IVF procedures (16). Sixty-nine percent of the cultured oocytes had cleaved 3 days after fertilization and 28% developed to the blastocyst stage. These percentages correspond with the results obtained with oocytes from the slaughterhouse during the same time period.

Tabel 1. Preliminary results with the new ovum pick-up (OPU) needle guidance system

OPU session	number of cows	number of aspirated follicles	number of collected oocytes	oocyte recovery rate	% cleavage 3 dpi	% EB 8 dpi
1	1	5	3	60%	100% (3/3)	0%
2	1	2	2	100%	0%	0%
3	4	10	6	60%	67% (4/6)	33% (2/6)
4	4	12	5	42%	100% (2/2) <sup>c</sup>	50% (1/2)
5	1	5	1	20%	0%	0%
6	1	2	0	0%	0%	0%
7	8	69 <sup>a</sup>	16	23%	44% (7/16)	19% (3/16)
8	4	29 <sup>b</sup>	6	21%	50% (3/6)	0%
9	2	8	5	63%	75% (3/4) <sup>d</sup>	25% (1/4)
10	4	18	7	39%	71% (5/7)	29% (2/7)
11	7	22	7	32%	71% (5/7)	14% (1/7)
12	1	4	1	25%	100% (1/1)	100% (1/1)
13	7	28	18	64%	78% (14/18)	17% (3/18)
14	6	21	8	38%	63% (5/8)	38% (3/8)
15	4	12	4	33%	100% (4/4)	75% (3/4)
16	5	21	14	67%	57% (8/14)	36% (5/14)
17	6	23	19	83%	94% (16/17) <sup>e</sup>	47% (8/17)
Overall results for 27 diff. cows		291	122	42%	69% (80/116)	28% (33/116)

a OPU session after FSH/LH stimulation

b OPU session 3 days after stimulated session

c 2 out of 5 oocytes were cultured

EB early blastocyst

dpi days post insemination

d 4 out of 5 oocytes were cultured

e 17 out of 19 oocytes were cultured



## DISCUSSION

The ultrasound guided retrieval of bovine oocytes has proved to be a reliable technique to provide oocytes from living cows (7,10) and its main advantage is its high degree of repeatability. In combination with good IVF and cryopreservation techniques, it can become a valuable alternative to classical embryo transfer.

To optimize the technique of OPU and to increase the recovery rate of oocytes it is important to reconsider the procedure used. An important part of the system are the needles being used (Baltussen, personal communication). The most important part is the point of the needle, which has to be as sharp as possible in order to pass the vaginal wall easily and to minimize damage to the ovaries. Long nondisposable needles become dull after use in two or three OPU sessions and need resharping frequently while a resharpened needle never regains its initial sharpness. The use of a disposable equivalent eliminates this problem by replacing a dull needle with a new one. Furthermore, disposable needles are very inexpensive and because of their small dead volume, they are very well suited for collection of small amounts of follicular fluid from individual follicles. However, a disadvantage of the use of disposable needles is the more complex needle guidance system required to permit changing of the needle, and its implications concerning the type ultrasonographic scanner used. Furthermore, the possibility that a cumulus-oocyte-complex (COC) remains behind in the needle-tubing system, necessitates rinsing of the tubing and examination of the rinsing fluid after each OPU session. Therefore, new systems should be of simple construction to permit easy changing of the needles and to prevent loss of oocytes. It is not possible to rinse the follicles with this new construction, but since bovine ovarian midcycle follicles are relatively small (5-10 mm), as compared to equine follicles, rinsing is probably not needed. A disadvantage of a rinsing system is its complexity, which results in several junctions and cavities in which the COC can be trapped. The gain in numbers of oocytes by rinsing follicles is probably offset by the loss of COC's due to the complexity of the tubing system.

The OPU success rate is partly measured by the recovery rate (RR), which is influenced by hormonal pretreatment of the animals (8,12), puncture frequency (4,15), vacuum level used (unpublished data) and operator experience (13,15). As a result RR's vary between 7% (13) and 69.6% (7), for different research teams. We obtained an overall recovery rate of 42 % with the new disposable guidance system, which may increase as it is used routinely.

When OPU is used routinely, application of short disposable needles is desirable from an economical and practical point of view. Use of disposable needles necessitates modification of the currently used OPU devices and adaptations for changing the needles. Combining the new needle guidance system with a MAP transducer results in a highly stable construction that permits the use of short disposable 19-g needles and the easy manipulation of the ovaries within the scanned area. The first results obtained with this new OPU system are encouraging. The possible effect on embryo quality concerning the loss of cumulus cells and the optimization of suction power need further investigation.

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